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Neuromodulatory role of *Bacopa monniera* extract on Cerebral Cortex Structural Damage and Oxidative Stress in Aluminum Intoxicated Rats

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Abstract

Decreased antioxidant status in brain, particularly in cerebral cortex region leads to tissue damage, and plays a key role in the progression of cognitive impairment and neurodegenerative diseases.

Aim: The present study attempts to assess the protective potential of *Bacopa monniera* ethanolic extract (BME) against aluminum mediated neurotoxicity in the cerebral cortex of rats.

Methods: Rats were divided into four groups i.e control (CON), aluminum maltolate (AlM) treated, *Bacopa monniera* ethanolic extract (BME) treated and combination of aluminum plus BME (AlM+BME) treated groups, each group contains six rats, the oral dosage was given for 4 weeks.

Results: Results shows that significant ($P<0.05$) decline in the activity of endogenous antioxidant enzymes including, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and elevated thiobarbituric acid reactive substance (TBA-RS) levels associated with AlM. However co-administration of BME significantly ($P<0.05$) restored these antioxidant enzymes against AlM induced reduction. BME treatment resulted in reduction of TBA-RS levels thus inhibits the lipid peroxidation. BME prevents the AlM neurotoxicity and it was clearly observed at light microscopic and ultrastructural level through electron microscopic images, indicative of its neuroprotective effect.

Conclusion: These findings suggest that BME is capable in preventing the AlM induced cerebral cortex damage by decreased lipid peroxidation and improved antioxidant capacity.

Keywords: Aluminum toxicity, *Bacopa monniera*, TBA-RS, antioxidant enzymes, Light microscopy, Transmission electron microscopy

INTRODUCTION

Aluminum has been suggested as a potential neurotoxic metal implicated in the progression of a number of neurodegenerative diseases including Alzheimer's disease [1,2,3] . The most usual aluminum exposure for the general population is through the diet mainly from dietary additives [4, 5, 6] . The high amount of aluminum in processed foods is due to the presence of aluminum additives used as rising agents, dyes, anticaking agents and pH adjusting [7] . Although aluminum is poorly absorbed it has been shown that some aluminum compounds such as maltolate, ascorbate, succinate, lactate and citrate are more easily absorbed, specifically maltolate is a common component of human diet, it is by product formed during sucrose pyrolysis or thermal degradation of starch [8] and it can be found in coffee, soy bean, baked cereals and caramelized and browned foods [9] as it does not form insoluble precipitates of aluminum hydroxide at physiological pH and this aluminum complex is advantageous for use in *in vitro* mechanistic studies. There is a potential for aluminum maltolate (AlM) to form in the gastro intestinal tract because of high affinity of maltolate for aluminum and also maltolate may facilitate the entry of aluminum into brain [10] thereby increasing a potential neurotoxicity, therefore investigating the enhanced toxicity of AlM is relevant to human health. *Bacopa monniera Linn* belongs to family scrophulariaceae, it is a small creeping herb commonly known as Brahmi found in wet, damp and marshy areas of tropical regions [11] . It has been used in the ayurvedic medicine for centuries and has antioxidant properties that may offer protection from free radical damage.

Based on this background, present study was designed to investigate the neuroprotective effect of BME against AlM induced structural damage associated with oxidative stress in cerebral cortex of albino rats.

MATERIALS AND METHODS

Chemicals

Al $(NO_3)_3.9H_2O$, Maltol, NBT, TBA were purchased from the Sigma Chemical Company (USA). H_2O_2, BSA and other chemicals were obtained from Merck and Himedia Chemical Companies.

Preparation Bacopa monniera ethanolic extract

Bacopa monneria plants were collected from Thummala Gunta fields, Tirupati, Andhra Pradesh, India. Plants were identified and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. (Voucher No.1213). The plants were dried in shade and then powdered. The powdered plant material was taken in a conical flask and extracted with 90% ethanol in a mechanical shaker with temperature control (Room temperature) and constant stirring at 200 rpm. It was left for 24 h and solids were filtered using Whatman No.1 filter paper (Raaman 2006) [12]. The extraction was repeated three times until complete extraction. The residue obtained after

removing the solvent, dried in vacuum and macerated with acetone to give free flowing powder.

Successive Soxhlet extraction

Soxhlet equipment was used in this study. Powdered plant material was extracted with ethanol in Soxhlet apparatus (Raaman 2006) [12].

Animals

Male adult albino rats of 3 months age with body weight 200 ±50gm were used. The rats were procured from an authorized vendor (Sri Venkateswara Enterprises Bangalore, India), randomized six per group in polypropylene cages (47x34x20cm) containing sterile paddy husk as bedding and maintained at 22-25˚C under a bell regulated light and dark (12h:12h). The rats were fed on standard rat chow (Sai Durga feeds and foods, India) and water ad *libitum.* The
study design and protocols were approved by the Institutional Animal Ethics Committee of Sri Venkateswara University, India (No. 03/2012-13/(i)a/CPCSEA/IAES/SVU/PJD-RPC/dated 1/2/2012).

Experimental design

Animals were equally randomized to four groups of 6 animals each

(a) Group-I: Control: administered with (0.9%) saline solution.
(b) Group-II: AlM treated rats: AlM was administered orally at a dose of 100mg/kg b.w.
(c) Group-III: BME treated rats*:* BME was administered orally at a dose of 40mg/kg b.w.
(d) Group-IV: AlM and BME treated rats: AlM administered simultaneously with BME

The dosage period was for four weeks.

Tissue collection and preparation of tissue homogenates

Six rats of each group were sacrificed and dissected after the treatment period. Brains were quickly taken out and cooled in a deep freezer. Cerebral cortex of the brains of 6 rats rapidly dissected out on ice plate and pooled for biochemical assays and histopathological analyses. Tissue samples were homogenized in 50 mM Tris (pH 7.4) with a Potter-Elevehijam type homogenizer fitted with Teflon plunger. The homogenate was diluted 1:10 (with Tris, pH 7.4 buffer) and centrifuged at 6000 rpm for 5 min in a refrigerated centrifuge. All procedures were carried out in ice cold conditions.

Determination of thiobarbituric acid reactive substances (TBA-RS)

Thiobarbituric acid reactive substances (TBA-RS) an index of lipid peroxidation, was estimated by Ohkawa et al. 1979 [13]. The amount of TBA-RS was determined spectrophotometrically at 532 nm. Values are expressed as micromoles of TBA-RS per mg protein.

Mesurement of antioxidant enzyme activities

Superoxide dismutase activity (SOD)

SOD activity was measured as the inhibition of photoreduction of nitroblue tetrazolium (NBT) by the Misra and Fridovich, 1972 [14]. Results were expressed as unit of SOD /min / mg protein.

Catalase activity (CAT)

CAT was assayed spectrophotometrically using the method of Aebi et al., 1984 [15]. The decrease in absorbance was observed for 60s at every 15s interval. Catalase activity is expressed as µmol of H_2O_2 decomposed/min/g tissue.

Glutathione peroxidase (GPx)

Glutathione peroxidise activity was measured using the method of Flohe and Gunzler 1984 [16]. GPx activity is expressed as µmol/mg/min.

Protein estimation

Protein estimation was done by Lowry et al., 1951 [17]. Bovine serum albumin was used as standard and the colour developed was read at 660nm. The protein content is expressed as mg/ gm wet wt of the tissue.

Statistical analysis

Results are expressed as mean ±S.D (standard deviation of the mean). Data were analyzed using the one way analysis of variance followed by Scheffe's contrast. The 0.05 level of probability was used as the criterion of significance in all cases.

Light Microscopy

Tissues were isolated from control and experimental treated rats. They were gently rinsed with physiological saline solution (0.9% NaCl) to remove blood and debris adhering to the tissuesand fixed in 15% formalin for 24 hrs. The fixative was removed by washing through running tap water overnight. After dehydrating through a graded series of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Sections were cut into 6µ thickness and stained with hematoxylin and counter stained with eosin (dissolved in 95% alcohol). After dehydration and cleaning, sections were finally viewed under light microscope (Harris, 1900) [18].

Transmission electron microscopy (TEM)

The cerebral cortex of different groups were fixed in 2.5%-3% glutaraldehyde made in 0.1M phosphate buffer (pH-7.2) for 24 hr at 4ºC and post fixed in 2% aqueous osmium tetroxide in the same buffer for 2hr. Dehydrated in series of graded alcohols, infiltrated and embedded in araldite 6005 resin or spur resin. Ultra thin (50-70nm) sections were made with a glass knife on ultra microtome (Leica ultra cut UCT-GA-D/E-1/00), mounted on copper grids and stained with saturated aqueous uranyl acetate and counter stained with Reynolds lead citrate (Bozzola and Russell 1998) [19]. These sections were finally viewed under transmission electron microscope (Hitachi, H-7500 from Japan).

Statistical analysis

Results are expressed as mean ±S.D (standard deviation of the mean). Data were analyzed using the one way analysis of variance followed by Scheffe's contrast. The 0.05 level of probability was used as the criterion of significance in all cases.

RESULTS

Effect of BME on TBA-RS levels induced by aluminum cerebral cortex of rat

In the present study, AlM exposure for four weeks significantly enhanced TBA-RS levels, lipid peroxidation markers in the cerebral cortex of albino rats compared to control group. Whereas co-administration of BME along with ALM significantly inhibited the TBA-RS levels compared to ALM alone treated rats (Fig.1).

Protective effect of BME on SOD, CAT and GPx activities

AlM treated group showed significant reduction in the SOD, CAT and GPx activities in the cerebral cortex compared to the control group, whereas AlM+ BME treated group showed a significant increase in SOD, CAT and GPx activities compared to the AlM treated group (Fig.2, 3 and 4).

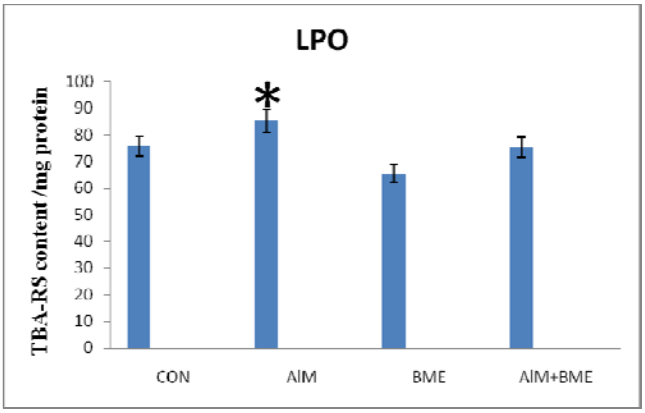


Fig.1 Effect of *BME* on Thiobarbituric acid reactive substance (TBARS) levels in cerebral cortex of rats exposed to AlM. * significant compared to control. Results are expressed as mean ±S.D (standard deviation of the mean). The level of probability less than 0.05 used as the criterion of significance in all cases.

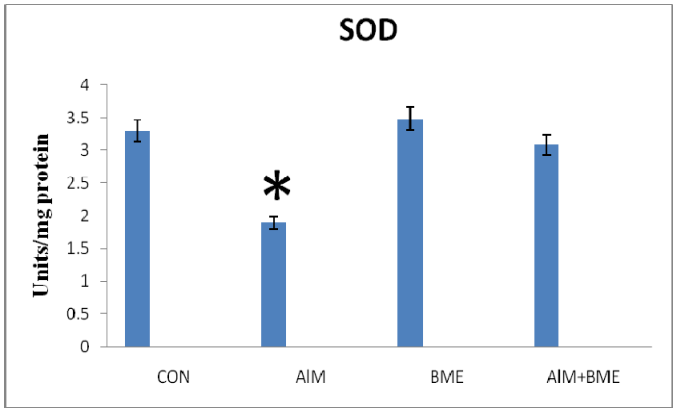


Fig.2 Effect of *BME* on Superoxide dismutase (SOD) levels in cerebral cortex of rat exposed AlM. * significant compared to control Results are expressed as mean ±S.D (standard deviation of the mean). The level of probability less than 0.05 used as the criterion of significance in all cases.

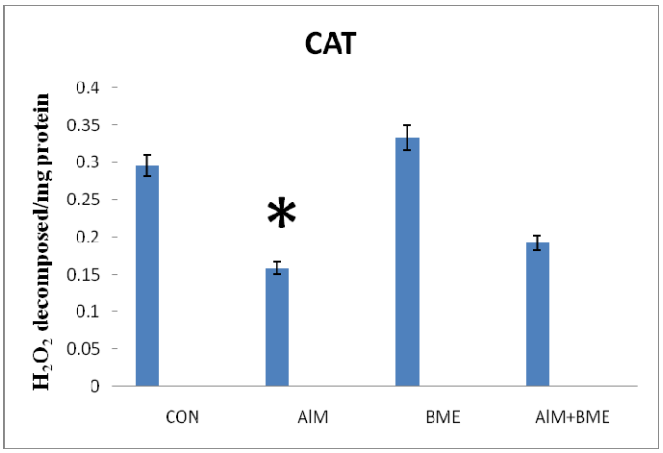


Fig.3 Effect of *BME* on Catalase (CAT) levels in cerebral cortex of rat exposed AlM. * significant compared to control Results are expressed as mean ±S.D (standard deviation of the mean). The level of probability less than 0.05 used as the criterion of significance in all cases.

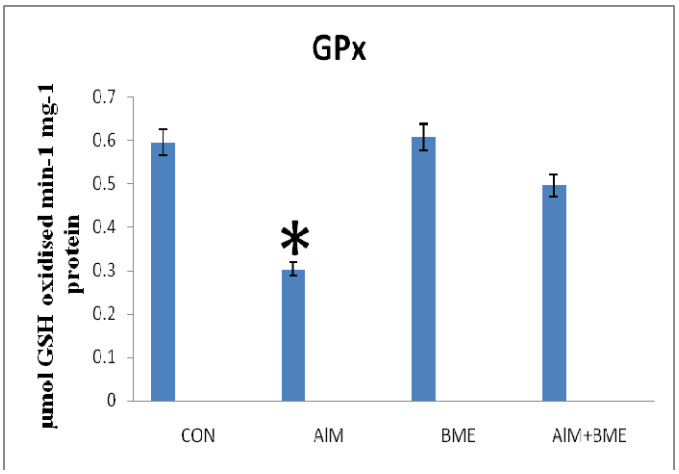


Fig.4 Effect of *BME* on Glutathione peroxidase (GPx) levels in cerebral cortex of rat exposed AlM. * significant compared to control Results are expressed as mean ±S.D (standard deviation of the mean). The level of probability less than 0.05 used as the criterion of significance in all cases.

Protective role of BME on aluminum induced histopathological changes in cerebral cortex of rat

We performed the histopathological studies by light microscope to evaluate the protective effects of BME treatment against Al-M-induced damage. Photomicrograph of cerebral cortex with Al intoxication showed Perivascular spaces (PVC) and degenerative changes in glial cells (DGGC) (Fig.5.b). These structural changes elucidate the impaired cerebral cortex function by Al exposure. The key findings of this study reveals that Al alone induced cerebral cortex damage was decreased by BME treatment. The degenerative changes occurred due to Al exposure was reversed by the co-administration of *Bacopa monniera* extract (Fig.5.d). BME treated rat's shows normal texture as control rats (Fig. 5.c).

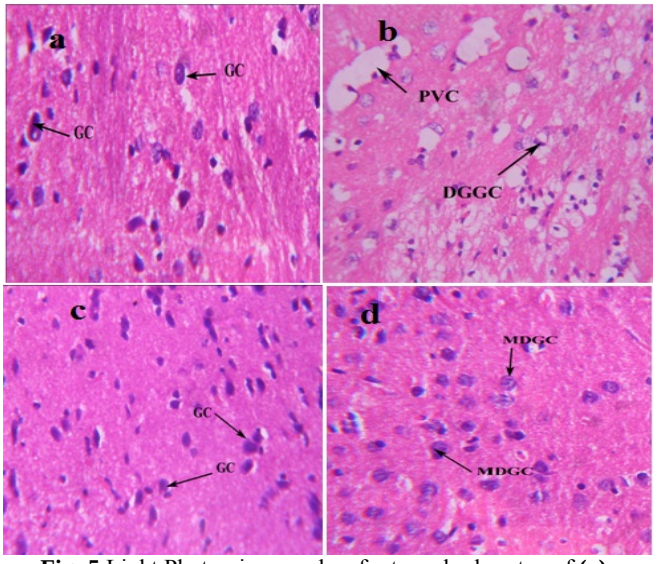
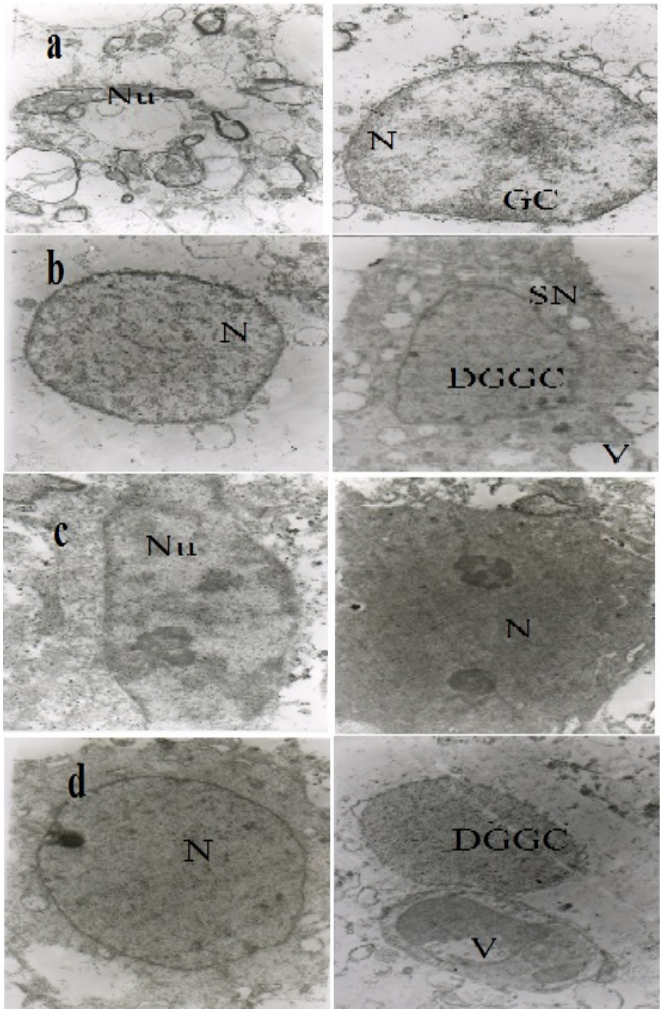


Fig: 5 Light Photomicrographs of rat cerebral cortex of **(a)** Control rat cerebral cortex showing glial cells (GC), **(b)** Aluminum treated cerebral cortex showing Perivascular spaces (PVC) and degenerative changes in glial cells (DGGC), **(c)** Bacopa treated rat cerebral cortex showing glial cells (GC) and **(d)** Aluminum and Bacopa treated rat Cortex showing mild degenerative changes in glial cells (MDGC). H &E. 400X

Role of BME on aluminum induced Ultrastructural changes in cerebral cortex of rat

We performed the ultrastructural studies by transmission electron microscopy to explore the therapeutic effect of BME against AlM-induced cerebral cortex damage. TEM micrograph of AlM treated cerebral cortex showed shrinken nucleus, vacuolation and degenerative changes in granular cell (Fig.6b). These structural changes elucidate the impaired cerebral cortex function by AlM exposure. The Present study reveals that AlM induced cerebral cortex damage was reduced by the co-administration of BME treatment (Fig.6d).



X 400 X 1200

Fig.6 TEM Photomicrographs of rat cerebral cortex of **(a)** control rat cerebral cortex showing neuron (Nu) prominent nucleus (N) and granule cells (GC). (**b)** AlM treated rat cerebral cortex showing shrinken nucleus (SN) , vacuolation (V) and degenerative changes in granular cell (DGGC). (**c)** *BME* treated rat cerebral cortex showing nucleus (N), and densely occupied supporting granular cell (GC). (**d)** AlM plus *BME* treated rat cerebral cortex showing swollen nucleus (SN), vacuolation (V) and mild degenerative changes in granular cell (MDGGC).

DISCUSSION

Increase in commercial and industrial applications of aluminum have been linked with the risk of Alzheimers's disease and other neuropathological diseases. As cerebral cortex plays an important role in memory, attention, perceptual awareness, thought, language and consciousness this region is particularly susceptible to Alzheimer's disease. In recent years, a number of reports demonstrated that Al administration increases lipid peroxidation in rat and mouse brain [20, 21, 22, 23, 24, 25, 26]. Exposure of Al led to marked increase in TBA-RS levels. These results are in consonance with Jyoti et al. (2007) [21] who reported that the Al administration causes increase in TBA-RS levels, an index of oxidative stress leading to elevation of free radicals and deterioration of cellular signal transduction in cortex. When the generation of free radicals overwhelms the antioxidant defence, lipid peroxidation of the cell membrane occur. The increase in lipid peroxidation might be due to accumulation of excess iron which may facilitate Fe catalyzed reaction results generation of reactive oxygen species (ROS) [27].

In the current study with administration of aluminum, the antioxidant enzymes such as SOD, CAT and GPx were reduced in the cerebral cortex. This observation is in agreement with the previous reports [28, 29, 27]. The reduced activity of SOD and CAT are responsible for cell damage and free radical production with Al exposure [30]. Buraimoh et al. (2012) [23] reported that cerebral cortex showed neuronal vacuolation and necrosis with the administration of Al. The antioxidant enzymes such as SOD and CAT plays an important role in detoxifying superoxide and hydrogen peroxide in the cells. The glutathione peroxidase system consists of several components which can effectively remove (hydrogen peroxide), certain drugs and chemicals and other reactive molecules from the cells. Some times because of toxic metals excess of free radicals will be generated which may not be removed by antioxidant system [31]. Kishore et al.

(2005) [32] reported that exposure of aluminium significantly decreases the brain glutathione levels. Existing reports indicated that bacosides are responsible for the antioxidant and tissue protective properties of BME [33, 34, 35, 36] . The data obtained by the present study illustrated, BME maintained the levels of LPO, SOD, CAT and GPx activities in the cortex nearly at control values. Accordingly, BME significantly ($p<0.05$) inhibited the increase in LPO levels and enhanced the SOD, CAT & GPx activities in cerebral cortex of AlM plus BME administrated rats. From the histopathological examination we observed perivascular spaces and degenerative changes in glial cells due to administration of AlM (Fig.5b), but these effect was reversed by BME treatment (Fig.5d). We performed the ultrastructural studies by transmission electron microscopy to explore the therapeutic effect of BME against AlM-induced cerebral cortex damage. TEM micrograph of AlM treated cerebral cortex showed shrinken nucleus, vacuolation and degenerative changes in granular cell (Fig.6b).These structural changes elucidate the impaired cerebral cortex function by AlM exposure. The Present study reveals that AlM induced cerebral cortex damage was reduced by the co-administration of BME treatment (Fig.6d). Ultrasructural studies expressed that AlM exposure induced loss of parkinje cells neurons and altered granular cell layer of the cerebral cortex. This is in consonance with the previous reports [36, 37] . Al causes histopathological lesions in cerebral cortex including neuronal degeneration as cytoplasmic vacuolization, hemorrhage, ghost cell and gliosis.

This corresponds well to the finding of Jyoti et al. (2007) [21] who proposed that Al neurotoxicity is mediated through oxidative damage and histopathological changes. BME is potential to counter this neurotoxicity. In conclusion, the results of the current study suggest that BME ameliorates cerebral cortex oxidative stress structural damage and act as effective antioxidant against the toxicity induced by AlM in rats.

CONCLUSIONS

The findings of the present study suggest that BME has beneficial effect in restoring antioxidant enzymes and inhibiting the TBA-RS levels as well as tissue damage against AlM toxicity. Hence BME was proven as more potential in preventing the oxidative stress and structural damage induced by AlM administration.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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